Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem

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[1] Understanding the controls over ecosystem-respired $\delta^{13}C$ ($\delta^{13}C_R$) is important for applications of isotope-based models of the global carbon budget as well as for understanding ecosystem-level variation in isotopic discrimination (Δ). Discrimination may be strongly dependent on synoptic-scale variation in environmental drivers that control canopy-scale stomatal conductance (G_c) and photosynthesis, such as atmospheric vapor pressure deficit (vpd) photosynthetically active radiation (PAR) and air temperature (T_{air}). These potential relationships are complicated, however, due to time lags between the period of carbon assimilation and ecosystem respiration, which may extend up to several days, and may vary with tissue (i.e., leaves versus belowground tissues). Our objective was to determine if relationships exist over a short-term period (2 weeks) between meteorological and physiological driving factors and $\delta^{13}C_R$ and its components, soil-respired $\delta^{13}C$ ($\delta^{13}C_{R\text{-}soil}$) and foliage-respired $\delta^{13}C$ ($\delta^{13}C_{R\text{-}foliage}$). We tested for these hypothesized relationships in a 250-year-old ponderosa pine forest in central Oregon, United States. A cold front passed through the region 3 days prior to our first sample night, resulting in precipitation (total rainfall 14.6 mm), low vpd (minimum daylight average of 0.36 kPa) and near-freeze temperature (minimum air temperature of 0.18°C ± 0.3°C), followed by a warming trend with relatively high vpd (maximum daylight average of 3.19 kPa). Over this 2-week period G_c was negatively correlated with vpd ($P < 0.01$) while net ecosystem CO_2 exchange (NEE) was positively correlated with vpd ($P < 0.01$), consistent with a vpd limitation to conductance and net CO_2 uptake. Consistent with a stomatal influence over Δ, a negative correlation was observed between $\delta^{13}C_R$ and G_c measured 2 days prior (i.e., a 2-day time lag, $P = 0.04$); however, $\delta^{13}C_R$ was not correlated with other measured variables. Also consistent with a stomatal influence over discrimination, $\delta^{13}C_{R\text{-}soil}$ was negatively correlated with G_c ($P < 0.01$) and positively correlated with vpd and PAR measured one to 3 days prior ($P = 0.01$ and 0.04, respectively). In contrast, $\delta^{13}C_{R\text{-}foliage}$ was not correlated with vpd or G_c, but was negatively correlated with minimum air temperature measured 5 days previously ($P < 0.01$) supporting the idea that cold air temperatures cause isotopic enrichment of respired CO_2. The significant driving parameters differed for $\delta^{13}C_{R\text{-}foliage}$ and $\delta^{13}C_{R\text{-}soil}$ potentially due to different controls over the isotopic content of tissue-specific respiratory fluxes, such as differing carbon transport times from the site of assimilation to the respiring tissue or different reliance on recent versus old photosynthate. Consistent with G_c control over photosynthesis and Δ, both $\delta^{13}C_{R\text{-}soil}$ and $\delta^{13}C_{R\text{-}foliage}$ became enriched as net CO_2 uptake decreased (more positive NEE, $P < 0.01$ for both). The $\delta^{13}C$ value of *Pinus ponderosa* foliage (−27.1‰, whole-tissue) was 0.5 to 3.0‰ more negative than any observed respiratory signature, supporting the contention that foliage $\delta^{13}C$ can be a poor proxy for the isotopic content of respiratory fluxes. The strong meteorological controls

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over G_c and NEE were associated with similar variation in $\delta^{13}C_{R\text{-}soil}$ but only minor variation in $\delta^{13}C_R$, leading us to conclude that $\delta^{13}C_R$ is not controlled solely by either canopy and belowground processes, but rather by their time-dependent interaction. *INDEX TERMS:* 1615 Global Change: Biogeochemical processes (4805); 1040 Geochemistry: Isotopic composition/chemistry; *KEYWORDS:* carbon isotopes, conductance, ecosystems, eddy correlation, old growth, *Pinus ponderosa*

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1. Introduction

[2] Concerns over rising concentrations of atmospheric CO_2 ($[CO_2]$) and subsequent effects on global warming have lead to aggressive use of new techniques to resolve the global carbon budget [*Canadell et al.*, 2000]. Among these techniques, measurements of the stable isotope composition of atmospheric CO_2 coupled with mass balance calculations, inverse global models, and biogeochemical models [i.e., *Ciais et al.*, 1995; *Battle et al.*, 2000; *Randerson et al.*, 2002a] are being utilized to constrain the terrestrial carbon sink as well as determine its regional location. However, observed variation in carbon isotope discrimination (Δ) and subsequent variation in the carbon isotopic composition of ecosystem respiration ($\delta^{13}C_R$) suggests that assuming constant values for these parameters may lead to inaccurate estimates of the land/ocean sink partitioning [*Fung et al.*, 1997; *Randerson et al.*, 2002b]. While a solid foundation exists for our understanding of Δ at the leaf-scale [*Farquhar et al.*, 1989], our theoretical and empirical knowledge of controls and variability over $\delta^{13}C_R$ is comparatively weak, thus causing uncertainty in our scaled estimates of $\delta^{13}C_R$.

[3] Recent work has shown significant within-site variation in $\delta^{13}C_R$ (up to 8.5‰) that appears to be driven by factors that influence leaf-level Δ [*Bowling et al.*, 2002]. In that study, a nonlinear relationship between $\delta^{13}C_R$ and vapor pressure deficit (vpd) was observed for a variety of coniferous forests along a precipitation gradient. *Ekblad and Högberg* [2001] found a similar link between $\delta^{13}C$ of soil-respired CO_2 ($\delta^{13}C_{R\text{-}soil}$) and atmospheric humidity. In both studies the isotopic response to humidity was in agreement with our traditional concept of a stomatal influence over Δ at the leaf-level (Figure 1, top). Increasing vpd typically causes a reduction in stomatal conductance [*Cowan*, 1994; *Hinckley and Braatne*, 1994; *Montieth*, 1995; *Oren et al.*, 1999] and consequently the supply of atmospheric CO_2 to the stomatal pore is reduced, thereby causing the ratio of atmospheric to internal, or sub-stomatal CO_2 (c_i/c_a) to decline (Figure 1). Reduced c_i/c_a subsequently forces a decrease in discrimination and hence an increase in the $\delta^{13}C$ of photo-assimilate (Figure 1, and see *Farquhar et al.* [1989] or *Ehleringer et al.* [1993] for a review of c_i/c_a controls on $\delta^{13}C$ of assimilated carbon).

[4] However, the relationship between $\delta^{13}C$ of photo-assimilate and $\delta^{13}C$ of respiratory fluxes may not be direct or immediate. An important result of the studies by *Ekblad and Högberg* [2001] and *Bowling et al.* [2002] is that respired $\delta^{13}C$ was correlated with humidity measured multiple days prior to the collection of the isotope data, rather than with humidity on the same day as the isotopic collections. This time lag indicates that the transport time of assimilate from foliage to the bulk of respiring tissue is relatively rapid, but not immediate. A similar, multiple-day time lag between assimilation and soil respiration was observed in the girdling study by *Högberg et al.* [2001]. As hypothesized in the bottom of Figure 1, the time lag may be associated with the transport time of carbon between the site of assimilation (foliage) and the respiring tissue. This time lag can be influenced by plant physiological factors including, but not limited to, transport distance, phloem temperature, sink or source strength, allocation of carbon between tissues or between respiration and dry matter production (Figure 1). Likewise, the time lag may be influenced by ecosystem level factors including, but not limited to, controls over soil respiration such as carbon transport from roots to fungi, hyphal transport time, microbial turnover, nitrogen availability, and soil temperature and moisture (Figure 1).

[5] In some ecosystems, however, little variation in $\delta^{13}C_R$ has been observed [*Flanagan et al.*, 1996; *Buchmann et al.*, 1998]. Such constancy of observed $\delta^{13}C_R$ may be due to limited sampling, a lack of variation in or insensitivity to environmental driving variables, a balancing effect of driving variables on Δ (i.e., if both stomatal conductance and assimilation rise in proportion causing constancy of c_i/c_a) or to a decoupling of Δ and $\delta^{13}C_R$. Decoupling may occur if the substrate used for respiration was not assimilated in recent days. For example, microbial respiration may switch from current (i.e., assimilated in the last few days) to relatively older (weeks to years old) photosynthate if cold temperatures reduce phloem transport rates, if freezing temperatures or (high vpd) cause stomatal closure and reduced photosynthetic rates [*Smith et al.*, 1984], or if soil moisture or oxygen availability becomes limiting to microbial metabolism [*Paul and Clark*, 1989]. In such a case, variation in Δ may not be observed in $\delta^{13}C_R$.

[6] Ecosystem-respired CO_2 results from the combined flux of CO_2 from the soil surface, foliage, stems and woody debris within the ecosystem. At the *Pinus ponderosa* site used in this study, ecosystem respiration is dominated by soil CO_2 flux (~76%) with the remainder dominated by foliar respiration [*Law and Ryan*, 1999]. Foliar and soil surface respiration should have different time lags between the moment of assimilation of a given carbon atom and the moment of respiration of the organic compounds containing that atom due purely to the transport distance from the site of photosynthetic assimilation to mitochondrial respiration. Simultaneous measurements of $\delta^{13}C_R$, $\delta^{13}C_{R\text{-}soil}$, and

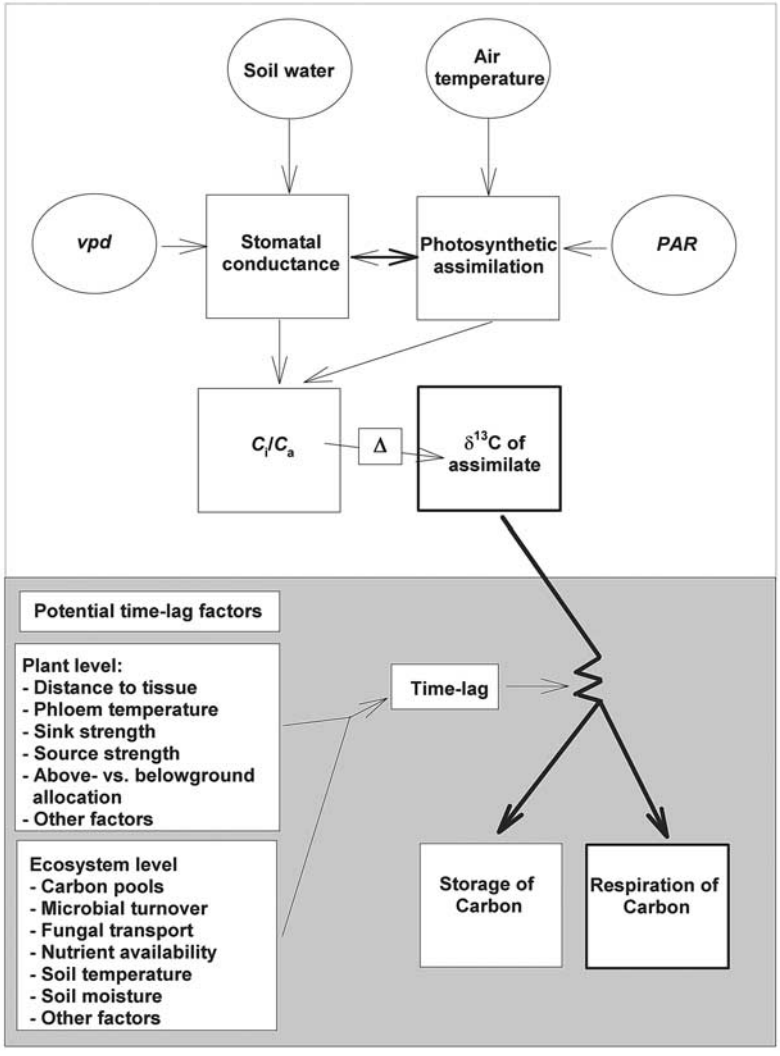

Figure 1. Theoretical representation of factors that may influence $\delta^{13}C_R$. (top) A simplified view of factors that may influence c_i/c_a, Δ and subsequent $\delta^{13}C$ of photo-assimilate. (bottom) Some factors that may influence the signature of $\delta^{13}C_R$ as well as the temporal lag between the time of carbon assimilation and respiration. The term "other factors" refers to factors that may yet be discovered.

$\delta^{13}C_{R\text{-foliage}}$ may provide insight into the controls over the time lag of $\delta^{13}C_R$ behind Δ, thus providing insight regarding the mechanisms that affect carbon allocation and transport.

[7] Combining measurements of the isotopic content of respiratory fluxes with estimates of net ecosystem exchange (NEE) can also provide insight into the potential relationships between $\delta^{13}C_R$ and ecosystem carbon flux. NEE is the ecosystem-scale balance of carbon assimilation and respiration,

$$\mathrm{NEE} = R - A, \tag{1}$$

where R is respiration and A is photosynthetic assimilation, both at the ecosystem-scale. Equation (1) is written such that more negative values indicate greater terrestrial CO_2 uptake. Canopy-averaged stomatal conductance (G_c) regulates both A and Δ and therefore should link NEE to $\delta^{13}C_R$. This prediction is described using the following equations. Leaf-level studies have shown that A is directly coupled to G_c [e.g., *Meinzer et al.*, 1993] because G_c controls CO_2 diffusion from the atmosphere to the stomatal pore, thereby controlling substrate availability to photosynthetic enzymes. In simple terms,

$$A \approx f(G_c). \tag{2}$$

In addition to its potentially dominant effect over NEE, G_c regulates Δ because G_c affects c_i,

$$c_i = c_a - \frac{A}{G_c}, \tag{3}$$

and c_i regulates Δ [*Farquhar et al.*, 1989],

$$\Delta = a + (b - a) \cdot \frac{c_i}{c_a}. \quad (4)$$

Last, the $\delta^{13}C$ composition of photosynthate results directly from Δ [*Brugnoli et al.*, 1988] such that

$$\delta^{13}C_R \approx \delta^{13}C_a - \Delta, \quad (5)$$

where $\delta^{13}C_a$ is the carbon isotope signature of atmospheric CO_2. Equation (5) is a simplified version of the ecosystem-scale discrimination equation suggested by *Buchmann et al.* [1998] and should hold for $\delta^{13}C_R$ if $\delta^{13}C_R$ results directly from $\delta^{13}C$ of photosynthate. In other words, equation (5) holds if canopy-scale Δ is the direct control over $\delta^{13}C_R$, for example, no fractionation occurs during phloem loading or respiration, and ecosystem-respired CO_2 is derived entirely from current photosynthate. Even though these assumptions are unlikely to be fully satisfied, we hypothesized that NEE and $\delta^{13}C_R$ are to some extent linked to G_c.

[8] The simple relationship between G_c, NEE, and $\delta^{13}C_R$ may be more complicated if $\delta^{13}C$ of photosynthate does not directly transfer to the isotopic fluxes of the various above and belowground components of the ecosystem, for example, due to time lags between canopy Δ and respired-$\delta^{13}C$. For example, the time lag between G_c and $\delta^{13}C_R$ should be longer than for G_c and NEE (if any lag exists) because $\delta^{13}C_R$ is associated with respiration and hence the lag is controlled by within-ecosystem carbon transport, whereas NEE is associated with both respiration and photosynthesis, the latter of which should be tightly coupled with G_c. This could be further confounded, however, if ecosystem respiration rate is correlated with time-lagged G_c.

[9] In this study, we measured $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$ nightly over a 2-week period with the objective of examining relationships between these fluxes and meteorological and physiological driving factors. Our two primary hypotheses were that (1) short-term fluctuations in vpd would be correlated with $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$, and (2) the mechanism underlying these correlations would be vpd regulation of G_c and subsequent G_c affects on $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$.

2. Methods

2.1. Site

[10] The study was conducted between days 179 and 191, 2001. The study site is a ponderosa pine (*Pinus ponderosa*) dominated forest located in the Metolius Research Natural Area near Sisters, Oregon (44°30'N, 121°37'W). The site is located at an elevation of 940 m on a nearly flat slope (2 to 6%). Ponderosa pine dominated forest extends for at least 12 km in all directions. The stand has two dominant age-classes of trees consisting of ~250-year-old trees and ~50-year-old trees, and a minor contribution (in regards to biomass) of saplings and seedlings. Understory vegetation is sparse. The canopy is open (leaf area index ~2.0 m^2 half surface area needles per m^2 ground), and vpd in the subcanopy is similar to that measured above the canopy [*Law and Baldocchi*, 1999]. The soil is a sandy loam and is low in nutrients. Climate at this site is characterized by warm, dry summers and wet, cool winters, with mean annual precipitation of 523 mm. This site is a member of the AmeriFlux network, and more extensive site details are given by *Law and Ryan* [1999], *Law et al.* [2001], and *Anthoni et al.* [2002].

2.2. Keeling Plots

[11] We used the Keeling plot approach [*Keeling*, 1958] to assess the isotopic composition of CO_2 in respiratory fluxes. This approach uses a two-component mixing model that consists of the carbon isotope ratio of CO_2 respired from all organisms within the forest and $\delta^{13}C$ of CO_2 in the background atmosphere. The intercept of a linear regression of $\delta^{13}C$ of atmospheric CO_2 versus $1/[CO_2]$ (where $[CO_2]$ is the mole fraction of CO_2) provides an estimate of $\delta^{13}C_R$. We used geometric mean (model II) regressions [*Sokal and Rohlf*, 1995]. Outliers were determined on each individual Keeling plot as described by *Bowling et al.* [2002]. We assumed no changes in $\delta^{13}C$ of the end-members during the sampling period for each individual Keeling plot. See *Pataki et al.* [2003] for more details on the application of Keeling plots in ecosystem science.

[12] Keeling plots were used to estimate $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_{R\text{-foliage}}$. $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ were sampled each night from day 179 to 191, and $\delta^{13}C_{R\text{-foliage}}$ was sampled each night from day 187 to 191. Year 2001 foliage from shoots neighboring those used for $\delta^{13}C_{R\text{-foliage}}$ was collected on day 179 for measurement of $\delta^{13}C$ of whole-tissue. Approximately three fascicles per branch were collected.

2.3. The Carbon Isotope Ratio of Ecosystem Respiration ($\delta^{13}C_R$)

[13] We sampled air from 0.2 m, 0.8 m and 11.4 m above the ground surface using Dekoron tubing (Dekoron/Unitherm Cable USA, Cape Coral, Fla.) placed on a scaffolding tower. We also had an inlet tube located above the top of the canopy (~33 m); however, previous sampling at this site showed no isotopic difference in air from the 11.4- and 33-m inlets. Air was pulled through magnesium perchlorate to remove water vapor prior to collection. Samples were then contained within 100-mL glass flasks with Teflon stopcocks (34–5671; Kontes Glass Co., Vineland, N. J.). Samples were collected nocturnally to avoid confounding influences of photosynthesis on $\delta^{13}C_R$. We typically collected air samples from 2100 to 2400 local time (LT) each night and obtained $[CO_2]$ ranges of 75 to 110 $\mu mol\ mol^{-1}$. Large $[CO_2]$ ranges improve estimates of $\delta^{13}C_R$ because the error around the regression intercept is negatively related to the $[CO_2]$ range [*Pataki et al.*, 2003].

2.4. The Carbon Isotope Ratio of Soil Respiration ($\delta^{13}C_{R\text{-soil}}$)

[14] We assessed $\delta^{13}C_{R\text{-soil}}$ using samples collected from a soil respiration chamber. A custom closed, dynamic soil chamber (70 cm × 70 cm × 10 cm tall, 49 L volume) with small internal fans (D249L, Micronel, Vista, Calif.) was placed in series with an infrared gas analyzer (LI-6262, Licor, Inc., Lincoln, Nebr.), a pump (UNMP50KNDC, KNF Neuberger, Inc., Trenton, N. J.), a magnesium perchlorate

water trap, and an assembly of six 100-mL sample flasks (connected to each other in parallel). First, all flask stopcocks were opened, and the pump was run for several minutes to flush the flasks and tubing with ambient forest air near the ground. Efforts were made to avoid contaminating the system with human breath. The chamber was then placed into a groove that had been previously cut through the litter layer to allow contact between the mineral soil and the chamber edge. The chamber was gently placed on the ground, then five of the flasks were closed sequentially in roughly 30 μmol mol^{-1} increments as $[CO_2]$ rose from near ambient to ~150 μmol mol^{-1} above ambient. Collection times were approximately 2 min. Three separate chamber locations were used each evening, shortly after dusk. The chamber locations were chosen to represent the three major stand-structure classes for this site: (1) open canopy with few, large trees, (2) closed canopy with dense stocking of small trees and few large trees, and (3) the boundary between the first and second classes. The soil chamber locations were approximately 75 m from the site of flask collection for $\delta^{13}C_R$. Nightly comparison of arithmetic averages of Keeling plot intercepts from the three chamber locations versus intercepts derived by pooling all soil chamber data for a single Keeling plot showed no significant differences, so data were pooled from all three soil chambers to generate a single Keeling plot for each night.

2.5. The Carbon Isotope Ratio of Foliage Respiration ($\delta^{13}C_{R\text{-foliage}}$)

[15] We refer to this measurement as $\delta^{13}C_{R\text{-foliage}}$; however, both woody stem tissue subtending the foliage and the foliage itself were included in the samples. Foliage and stems of two, 250-year-old ponderosa pine trees was accessed using a scaffolding tower. The samples were located approximately 25 m above the ground surface, and 125 m horizontally from the site of flask collection for $\delta^{13}C_R$. Ponderosa pine foliage is arranged in a spherical cluster around the end of the shoot. We wrapped entire foliage clusters in flexible bags (party balloons, Anagram International, Inc., Minneapolis, Minn.) with an internal layer of polyethylene. These bags have been tested for isotopic integrity and show no effect on $\delta^{13}C$ of gas samples after 60 min of gas residence within the bags (an order of magnitude longer than our samples resided within the bags). Details on the bags and isotopic tests are given by *Bowling et al.* [2003]. We cut holes in the bottom of the bags large enough for the foliage and attached the cut end of the bag to the shoot using putty (between the bag and shoot) and bungee cords wrapped on the outside of the bag/putty/shoot structure. Bags were attached to the branch just moments before sampling began and were removed after sampling completion. A small fan and inlet and outlet tubes (Dekoron) were placed within the bag. The tubes were run down the tower to the pump located on the ground surface. Samples were collected using a similar six-flask system as described for $\delta^{13}C_{R\text{-soil}}$. A single foliage cluster was measured every night, and on three nights (near the beginning, middle and end of the experiment) we measured five foliage clusters. All branches were located on the southside of the trees and the foliage was therefore "sun" foliage.

[16] Although this forest has a roughly equal amount of canopy leaf area contributed by the younger (50-year-old) age class, we were forced to constrain our $\delta^{13}C_{R\text{-foliage}}$ measurements to the old trees due to time, sample size, and access constraints. Therefore, our $\delta^{13}C_{R\text{-foliage}}$ measurements cannot be considered representative of the entire forest canopy.

2.6. Laboratory Analyses

[17] We measured carbon isotope ratios of flask samples on a continuous-flow isotope ratio mass spectrometer (IRMS; Finnigan MAT 252 or DELTAplus, San Jose, Calif.) as described by *Ehleringer and Cook* [1998]. Precision for $\delta^{13}C$ was determined daily by comparison to known standards and averaged 0.13‰ (standard deviation). Corrections for the presence of ^{17}O were applied. CO_2 was separated from N_2O by gas chromatography before analysis. Foliage tissue was ground to number 20 mesh and 2- to 20-mg samples were combusted and analyzed for $\delta^{13}C$ on an IRMS (deltaS, Finnigan MAT). Measurement precision for organic samples was 0.2‰. All $\delta^{13}C$ values are reported relative to the international PDB standard. Flask $[CO_2]$ was measured using the method of *Bowling et al.* [2001], and World Meteorological Organization CO_2 standards were used. Measurement precision was ± 0.2 μmol mol^{-1}.

2.7. Meteorological, Micrometeorological, and Eddy Correlation Measurements

[18] We collected meteorological and micrometeorological measurements at half-hourly intervals during the experiment. Measured parameters included air temperature, relative humidity, soil temperature, soil water content, photosynthetically active radiation, and rainfall. The eddy covariance method was used to determine half-hourly fluxes of CO_2 and water vapor above the forest canopy. Details of the meteorological and eddy covariance measurements are described by *Law et al.* [2001] and *Anthoni et al.* [2002].

2.8. Canopy Stomatal Conductance

[19] Mean midday canopy stomatal conductance (G_c) was estimated with a simplified form of Penman-Monteith equation [*Jarvis and McNaughton*, 1986] where whole tree sap flux measurements averaged between 1100 and 1300 LT were used to determine canopy transpiration. See work by *Irvine et al.* [2002] for more details.

2.9. Statistical Analyses

[20] We conducted correlation analyses to test our two primary hypotheses, that $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$ were coupled to vpd and that this relationship was due to G_c affects on discrimination (see section 1). We also used correlation analyses to determine the relationships between $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$, as well as relationships between these isotopic signatures and other variables expected to influence Δ or respiratory processes, including vpd, T_{air}, T_{min}, T_{soil}, PAR, θ, and NEE. Because correlations between these variables and $\delta^{13}C$ of respiratory fluxes may be lagged in time due to a delay between the time a given carbon atom is assimilated and respired, we conducted the correlations over a range of time lags. To do this, we

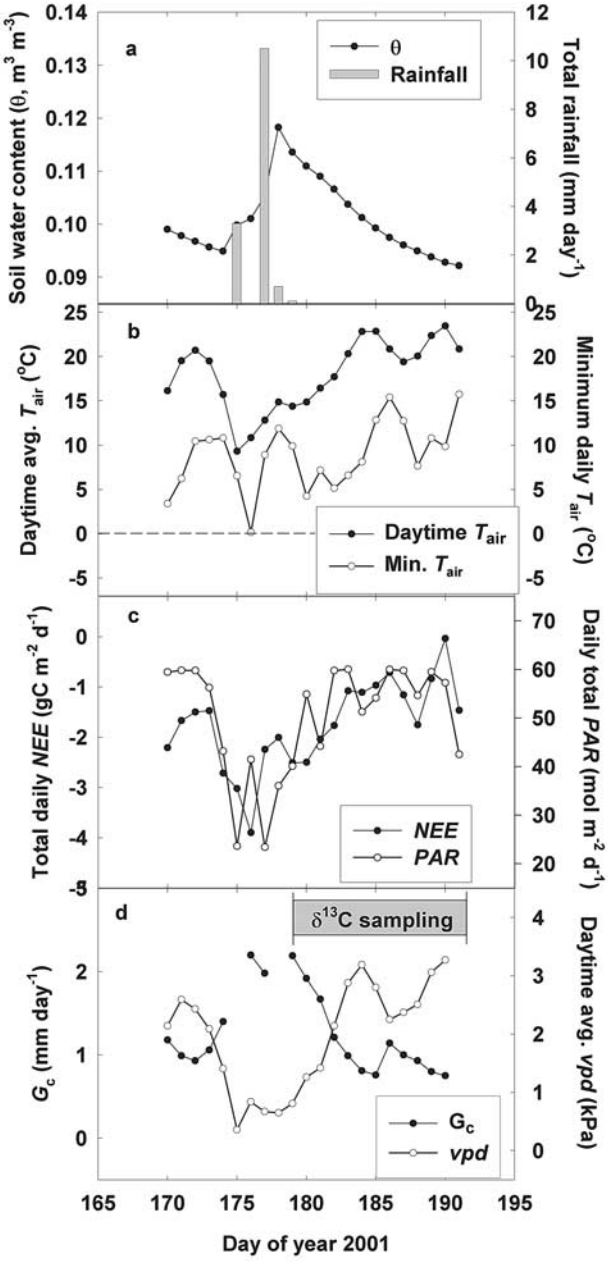

Figure 2. Meteorological and physiological data for days 170–191, 2001. (a) Average daily rainfall (solid bars) and θ (0 to 30 cm depth, solid circles). (b) Daytime T_{air} (solid symbols) and minimum T_{air} (open symbols). T_{air} reached a nocturnal minimum of 0.18°C ± 0.3°C on day 176. Zero Celsius is indicated by the dashed line. (c) The 24-hour total NEE (solid symbols) and daylight total PAR (open symbols). (d) Average daily G_c (solid symbols) during daylight periods. Daylight period vpd is shown as the open symbols. G_c data are omitted on days 175 and 178 because the canopy was wet on those days making those estimates suspect. The period of flask sampling for isotopic analyses is indicated by the shaded bar in Figure 2d.

calculated averages of a given independent factor from 1 to 5 days, and then shifted these averages back in time by zero to 15 days (a subset of these results are reported). See work by *Bowling et al.* [2002] for a more detailed description of lag analysis. SYSTAT 10.0 was used for statistical analyses.

3. Results

3.1. Meteorological and Physiological Patterns

[21] A cold front passed through central Oregon on days 175 through 178 (June 24–27), producing precipitation totaling 14.6 mm and increasing θ by 0.03 m^3 m^{-3} (Figure 2a). The minimum T_{air} (half-hourly average) during the cold-front was 0.18°C (± 0.3°C) (Figure 2b). Total daily PAR was reduced substantially by the cloud-cover during the storm, and this reduction in PAR was associated with high rates of net CO_2 exchange (more negative NEE, Figure 2c). The large net uptake on days 175 to 179 was associated with high G_c in conjunction with relatively low vpd (Figure 2d). As vpd increased from day 180 to 190, both G_c and net CO_2 uptake declined. G_c and NEE were strongly correlated, with high values of G_c nonlinearly associated with more negative NEE (i.e., more net CO_2 uptake, $P < 0.01$, data not shown). The G_c data are omitted on days 175 and 178 because the canopy was wet on those particular days and sapflow-based estimates of G_c are erroneous when the canopy is wet. Therefore, analyses with G_c data were conducted without data from days 175 and 178.

3.2. The $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$

[22] The isotopic contents of respiratory fluxes each day are shown in Figure 3. Observed $\delta^{13}C_R$ varied from −25.1 to −25.8‰, $\delta^{13}C_{R\text{-soil}}$ varied from −23.8 to −24.7‰, and $\delta^{13}C_{R\text{-foliage}}$ ranged from −23.4 to −26.6‰. Neither $\delta^{13}C_R$ nor $\delta^{13}C_{R\text{-foliage}}$ showed a time-trend over the duration of our measurements (regression $P = 0.66$ and 0.63, respectively). The $\delta^{13}C_{R\text{-soil}}$ was positively correlated with day of

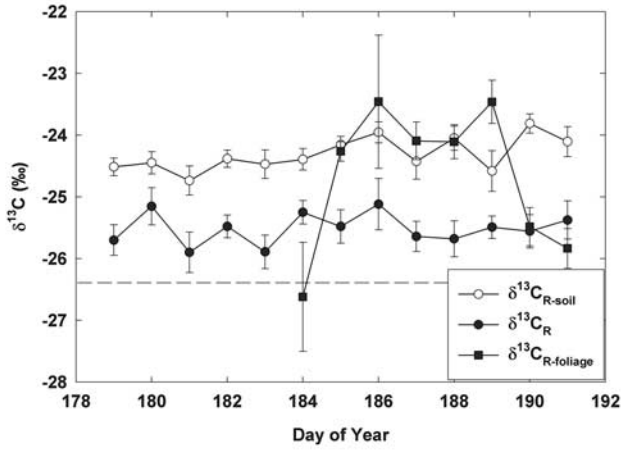

Figure 3. Observed $\delta^{13}C_R$ (solid circles), $\delta^{13}C_{R\text{-soil}}$ (open circles) and $\delta^{13}C_{R\text{-foliage}}$ (solid squares) versus day of year, 2001. The dashed line is $\delta^{13}C$ of whole-tissue foliage collected from old pine trees on day 179. Error bars represent standard errors.

Table 1. Coefficients of Determination (r^2) From Linear Regression Analysis of $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$ Versus Environmental and Physiological Driving Factors[a]

Component	vpd	T_{air}	T_{min}	T_{soil}	PAR	θ	G_c	NEE
$\delta^{13}C_R$	0.01	0.06	0.06	0.05	0.03	0.02	0.00	0.01
$\delta^{13}C_{R\text{-soil}}$	0.19	0.20	0.20	0.45[b]	0.04	0.39[b]	0.25[c]	0.3[b]
$\delta^{13}C_{R\text{-foliage}}$	0.06	0.05	0.06	0.01	0.49[b]	0.01	0.28	0.04

[a]All regressions presented in this table were done using a zero-day lag and a 1-day average. For example, $\delta^{13}C_R$ was correlated with single-day average vpd from the day of flask collection. All presented correlations were positive.
[b]Regression significance P = 0.05.
[c]Regression significance P = 0.10.

year (r^2 = 0.38, P = 0.03). Observed $\delta^{13}C_R$ was 1.2 permil; more negative than $\delta^{13}C_{R\text{-soil}}$ on average, and 0.85‰ more negative than $\delta^{13}C_{R\text{-foliage}}$ on average. The $\delta^{13}C$ of foliage tissue collected on day 179 was −27.1‰ (Figure 3).

[23] Observed $\delta^{13}C_R$ was not correlated with $\delta^{13}C_{R\text{-soil}}$ or $\delta^{13}C_{R\text{-foliage}}$. The best fit was between $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ (1-day lag, 1-day average, r^2 = 0.14, P = 0.20, n = 13). A correlation was observed between $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_{R\text{-foliage}}$, however, the correlation was strong due to clustering of four points at one end of the regression line and a single point at the other end (3-day lag, 1-day average, r^2 = 0.61, P = 0.03, n = 5).

[24] Coefficients of determination between $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, or $\delta^{13}C_{R\text{-foliage}}$ and meteorological and physiological variables expected to influence them are shown in Tables 1 and 2. In Table 1, the correlation analyses included regressions of $\delta^{13}C$ of respiratory fluxes versus the parameter of interest measured that same day (a zero-day lag) and with single-day averaging. Table 2 shows the results of regressions with the same independent and dependent factors but with lags ranging from zero to 15 days, and with averaging periods of 1 to 5 days. The direction of the relationships (positive versus negative) and significance of the regressions are also noted. Comparison of Tables 1 and 2 shows that, in general, relationships between isotopic signatures of respiration and driving parameters are quite poor when no lag period is accounted for. Only two regression combinations, $\delta^{13}C_{R\text{-soil}}$ versus θ and $\delta^{13}C_{R\text{-foliage}}$ versus PAR, exhibited the highest coefficients of determination for zero-day lags with 1-day averages (Table 1). All other regression combinations exhibited improved statistical fits if the driving parameter from a few days prior to the flask collections was used rather than from the day of the flask collection.

[25] Even after determining the appropriate lag and averaging periods, relatively poor correlations between $\delta_{13}C_R$ and measured driving parameters were observed, with only a significant relationship (P = 0.04) observed with G_c, and a marginally significant relationship (P = 0.08) with PAR (Table 2). Observed $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_{R\text{-foliage}}$ were more strongly correlated with measured driving parameters than $\delta^{13}C_R$. The $\delta^{13}C_{R\text{-soil}}$ correlations were generally noisy ($r^2 \sim$ 0.24 to 0.48), but were also relatively significant. The $\delta^{13}C_{R\text{-soil}}$ correlations with the tested parameters were all consistent with a stomatal control over Δ, including positive relationships with vpd, temperature, and PAR and negative relationships with θ and G_c. Observed $\delta^{13}C_{R\text{-foliage}}$ exhibited mixed results; being strongly, negatively correlated with minimum air temperature measured 5 days previously, but positively correlated with PAR measured the same day (Table 2). Shorter time lags were observed for $\delta^{13}C_{R\text{-soil}}$ than $\delta^{13}C_{R\text{-foliage}}$. Both $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_{R\text{-foliage}}$ were positively correlated with NEE, indicating that reduced net CO_2 uptake was correlated with isotopically enriched respiratory fluxes.

[26] We also assessed if there was a common lag/averaging combination that provided consistently high statistical fits for both $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$. We expected that these two isotopic signatures should share common lag/averaging periods because belowground respiration is the dominant respiratory flux in this ecosystem [*Law and Ryan*, 1999]. Both signatures shared relatively high statistical fits with PAR; a 4-day lag, single-day average, and 2-day lag, 2-day average both gave relatively strong fits for both components (data not shown). However, for no other driving parameter did we observe lag/averaging combinations that were shared by $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_R$.

[27] The results of our explicit hypothesis test that $\delta^{13}C$ of respiratory fluxes is related to short-term variation in vpd is shown in Figure 4. Figure 4a shows the previously observed relationship between vpd and $\delta^{13}C_R$ during periods when air temperatures were above 0.2°C (shown as a solid line) and when air temperatures were below 0.2°C (shown as the circled area) along with measurements from the present study (the solid line and circled area are from the work of *Bowling et al.* [2002]). Because no significant time lag was observed between $\delta^{13}C_R$ and vpd in the present study, the data is plotted with no lag (i.e., vpd from day 180 is plotted versus $\delta^{13}C_R$ from day 180). Alternatively, plotting the data with a 5-day lag, as observed by *Bowling et al.* [2002], causes little change in the figure (data not shown). The measured $\delta^{13}C_R$ values do fall within the range observed by *Bowling et al.* [2002];

Table 2. Results of Lag Analysis of $\delta^{13}C_R$, $\delta^{13}C_{R\text{-soil}}$, and $\delta^{13}C_{R\text{-foliage}}$ Versus Environmental and Physiological Driving Factors[a]

Component	vpd	T_{air}	T_{min}	T_{soil}	PAR	θ	G_c	NEE
$\delta^{13}C_R$	0.10 (+7, 1)	0.11 (+,1, 1)	0.17 (+, 7, 1)	0.05 (+, 0, 1)	0.25 (+, 4,1)[b]	0.06 (+, 6, 2)	0.35 (−, 2, 1)[c]	0.15 (+, 7, 1)
$\delta^{13}C_{R\text{-soil}}$	0.46 (+, 1, 3)[c]	0.52 (+, 1, 1)[b]	0.24 (+, 1, 1)[b]	0.45 (+, 0, 1)[c]	0.46 (+, 3, 2)[c]	0.39 (−, 0, 1)[c]	0.48 (−, 1, 2)[c]	0.48 (+, 1, 1)[c]
$\delta^{13}C_{R\text{-foliage}}$	0.30 (+, 3, 1)	0.64 (−, 5, 2)[c]	0.71 (−, 5, 2)[c]	0.22 (+, 1, 1)	0.49 (+, 0, 1)[c]	0.38 (+, 7, 1)[b]	0.30 (−, 4, 1)	0.69 (+, 2, 2)[c]

[a]The coefficient of determination (r^2) is presented for each lag/average combination that provided the best fit. The sign of the relationship (plus or minus) is presented in parentheses along with the number of days lagged and number of days averaged. For example, 0.46 (+, 3, 2) is a positive correlation with an r^2 of 0.46 and is a 3-day lag with a 2-day average. A zero-day lag with a 1-day average (0, 1) is identical to saying that a climatic factor yesterday was regressed against last night's isotopic signature.
[b]Regression significance P = 0.10.
[c]Regression significance P = 0.05.

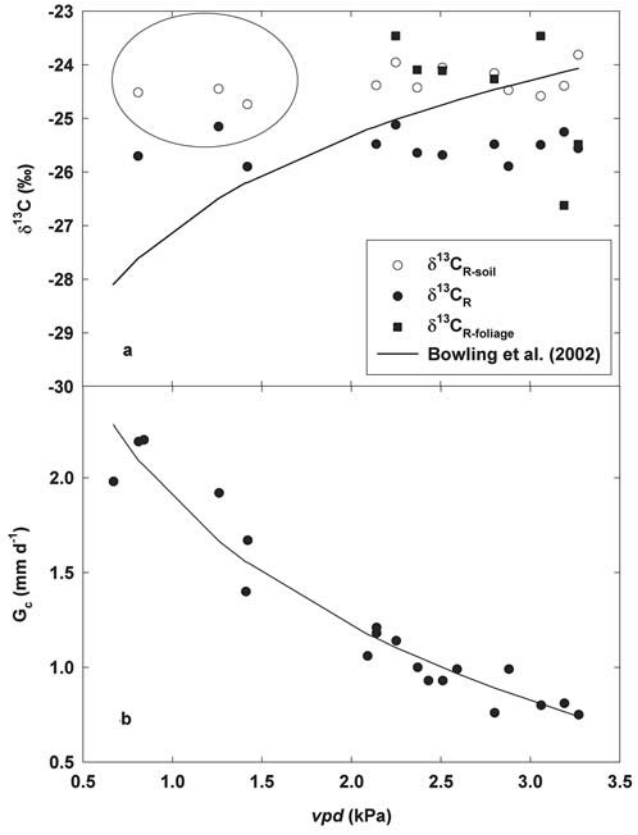

Figure 4. (a) Observed $\delta^{13}C_R$ (solid circles), $\delta^{13}C_{R\text{-soil}}$ (open circles), and $\delta^{13}C_{R\text{-foliage}}$ (solid squares) versus vpd. Because there was not a significant relationship between time-lagged vpd and $\delta^{13}C_R$, vpd on the *x* axis is not lagged. The solid line is the predicted relationship between $\delta^{13}C_R$ and vpd in the absence of freezing air temperatures, and the circled area is the prediction for $\delta^{13}C_R$ if freezing air temperatures occur [from *Bowling et al.*, 2002]. (b) G_c versus vpd (no lag). The line is $G_c = -0.97\ln(\text{vpd}) + 1.89$, $r^2 = 0.94$, $P < 0.01$.

however, they do not track the previously published vpd response. In contrast, G_c did exhibit the expected negative relationship with vpd (Figure 4b).

[28] Figure 5 shows the results of our explicit hypothesis test that G_c influences variation in isotopic content of respiratory fluxes. G_c is time lagged according to the best fit from Table 2 for $\delta^{13}C_R$ (2-day lag, Figure 5a) and $\delta^{13}C_{R\text{-soil}}$ (1-day lag, Figure 5b), and with no lag for $\delta^{13}C_{R\text{-foliage}}$ since that relationship was not significant ($P = 0.18$, Figure 5c).

4. Discussion

[29] The large ranges of vpd and G_c that occurred over the 2-week experiment (Figure 2) provided an ideal test of the hypothesis that short-term variation in vpd affects $\delta^{13}C_R$ via changes in canopy-level stomatal conductance. This test was conducted in order to determine (1) if the vpd-$\delta^{13}C_R$ relationship observed over 3 years and across a 250-km transect in Oregon [*Bowling et al.*, 2002] would also occur at a single site over a 2-week period, and (2) if canopy-level stomatal conductance was the mechanism controlling $\delta^{13}C_R$. While the overall results are inconclusive, the data shown in Figure 4a fail to support our first hypothesis. Over a 2-week period during the summer of 2001, $\delta^{13}C_R$ showed very little variation (Figures 3 and 4a) despite large changes in meteorological conditions and G_c (Figures 2 and 4b). The second hypothesis test, that G_c is related to $\delta^{13}C_R$, was marginally significant (Figure 5).

[30] Our failure to accept our first hypothesis, that $\delta^{13}C_R$ is affected by short-term variation in vpd, was due to relatively constant $\delta^{13}C_R$ over the 2-week period (Figures 3 and 4a).

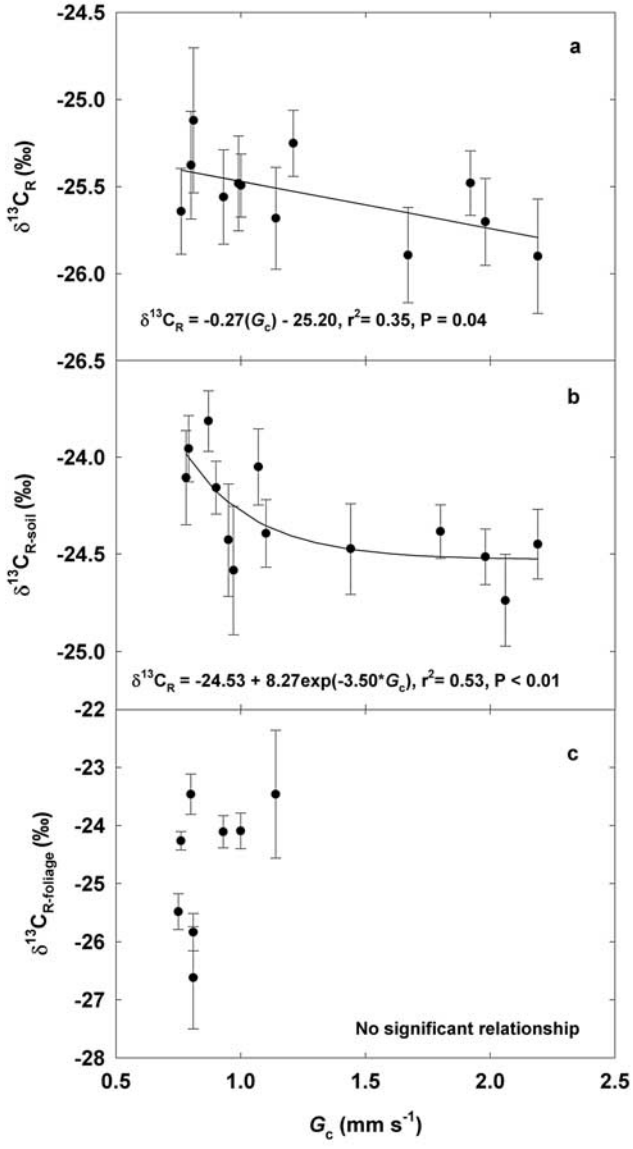

Figure 5. (a) Observed $\delta^{13}C_R$ versus G_c, where G_c is lagged by 2 days with a single-day average (Table 2). (b) Observed $\delta^{13}C_{R\text{-soil}}$ versus G_c, where G_c is lagged by 1 day with a 2-day average (Table 2). A nonlinear fit is shown for reference. (c) The $\delta^{13}C_{R\text{-foliage}}$ versus G_c, in which G_c is not lagged because no significant relationship was observed (Table 2). Error bars represent standard errors.

Of the theories proposed in section 1 to explain the lack of $\delta^{13}C_R$ variation observed in some other studies [i.e., *Flanagan et al.*, 1996; *Buchmann et al.*, 1998], we can exclude (1) limited sampling or (2) a lack of variation in or insensitivity to environmental driving variables. Our sampling was relatively intensive (nightly) over the 2-week period, and variation in environmental driving variables and resulting variation in G_c and NEE was large (Figures 2 and 4b). At least two alternative mechanisms exist that may contribute to the lack of variation in $\delta^{13}C_R$. From the plant physiological scale, a balancing effect of driving variables on c_i/c_a (i.e., if both G_c and assimilation rise in proportion causing constancy of c_i/c_a) or from the plant and ecosystem scales, to a decoupling of c_i/c_a and $\delta^{13}C_R$ (Figure 1).

[31] A balancing effect of driving variables that results in no variation in c_i/c_a could theoretically occur if freezing air temperatures are followed by a period of high vpd. The near freezing air temperature that occurred on day 176 (0.18°C, Figure 2b) could cause stomatal closure resulting in reduced conductance of CO_2 and hence reduced c_i/c_a [*Kaufmann*, 1976; *Fahey*, 1979; *Smith et al.*, 1984; *Kozlowski et al.*, 1991; *Strand et al.*, 2002] and subsequent enrichment of $\delta^{13}C_R$ at low vpd (see circled area in Figure 5a and see work of *Bowling et al.* [2002]). However, G_c showed no sensitivity to the cold air temperature on day 176 (Figures 2d and 4b), invalidating air temperature effects on G_c as the mechanism for enrichment of $\delta^{13}C_R$.

[32] Constant c_i/c_a may also occur if changes in G_c are mirrored by proportional changes in photosynthesis. We examined this theory by calculating c_i/c_a using G_c coupled with canopy photosynthesis data from the same 2-week period. Canopy photosynthesis was calculated using the measured daytime NEE data with respiration subtracted using a nocturnally based relationship between air temperature and respiration (P. Anthoni, unpublished data, 2003). Over the 2-week experiment, calculated c_i/c_a exhibited a wide range, from >0.70 to <0.50 μmol mol^{-1}, which is equivalent to $\delta^{13}C$ variation of <−28.0 to >−24.0‰. This suggests that $\delta^{13}C$ of photo-assimilates varied by 4.0‰ or more over the 2-week experiment, and subsequently, equivalent variation in $\delta^{13}C_R$ should have occurred if $\delta^{13}C_R$ is directly linked to $\delta^{13}C$ of photo-assimilate.

[33] A decoupling of c_i/c_a and $\delta^{13}C_R$ could occur via mechanisms operating at both the plant physiological and ecosystem scales. For example, $\delta^{13}C$ of photo-assimilates may be modified between assimilation and the time (and location) of respiration, or alternatively, tissue- or location-specific variation in respiratory substrate may have a canceling effect on the net isotopic signature respired at the ecosystem scale. It is well documented that c_i/c_a is directly related to $\delta^{13}C$ of photo-assimilates [*Brugnoli et al.*, 1988; *Lauteri et al.*, 1993; *Brugnoli et al.*, 1998], and furthermore, no fractionation occurs during mitochondrial respiration [*Lin and Ehleringer*,1997]. However, shifts in the isotopic composition of assimilates prior to respiration could occur [*Duranceau et al.*, 1999] leading to constant $\delta^{13}C_R$. At the ecosystem-scale, tissue-specific variation in respiratory substrate could occur for multiple reasons, including differential time lags for carbon transport to and respiration from foliage versus roots versus microbial biomass, changes in carbon allocation or sink strength, or a shift of respiration from labile- to recalcitrant carbon pools in the soil [*Schönwitz et al.*, 1986] such as may occur with changes in water content of the litter layer (B. Law, personal communication, 2003). We examined the theory that differences in $\delta^{13}C$ respired from aboveground versus belowground sources balanced at the ecosystem level by scaling the component fluxes of $\delta^{13}C_R$ using the following equation:

$$\delta^{13}C_R \cdot R_{ecosystem} = \delta^{13}C_{R-foliage} \cdot R_{-foliage} + \delta^{13}C_{R-soil} \cdot R_{-soil}, \quad (6)$$

where R stands for the fraction of total ecosystem respiration from the ecosystem (100%), foliage, or soil (the latter two summing to 100%). On the basis of conservation of mass, the sum of the right-hand side of equation (1) equals $\delta^{13}C_R \cdot R_{ecosystem}$. We used a range of values for $R_{-foliage}$ and R_{-soil}, ranging from 24 and 76% of $R_{ecosystem}$, respectively [*Law and Ryan*, 1999] to 50% and 50% of $R_{ecosystem}$, respectively. However, all scaling combinations of the component fluxes predicted $\delta^{13}C_R$ substantially more enriched than measured $\delta^{13}C_R$ (1.0 to 2.5‰). Therefore, we have little evidence of a decoupling of c_i/c_a and $\delta^{13}C_R$.

[34] The objective of our study was to examine temporal patterns of the isotopic signatures of CO_2 fluxes rather than to close an isotopic mass balance. Because of this objective and because of logistical constraints, we limited our foliage sampling to old trees. This exclusion of young trees may be responsible for the lack of mass balance because foliage tissue of young trees typically has more depleted $\delta^{13}C$ than old trees [*Yoder et al.*, 1994; *McDowell et al.*, 2002] and presumably more depleted $\delta^{13}C_{R\text{-foliage}}$. Measurement error in $\delta^{13}C_{R\text{-soil}}$ may also be responsible. CO_2 beneath the soil surface is enriched by up to 4.4‰ above soil surface CO_2 efflux due to fractionation during diffusion through the soil [*Cerling et al.*, 1991; *Davidson*, 1995]. Perturbation of pressure within the chamber headspace can cause advection of CO_2 out of the soil [*Fang and Moncrieff*, 1996; *Lund et al.*, 1999], potentially allowing enriched CO_2 to advect from the soil without the complete 4.4‰ fractionation. Such enrichment of CO_2 flux would result in a more positive Keeling plot intercept and hence a more positive estimate of $\delta^{13}C_{R\text{-soil}}$. Future work should address pump artifacts on $\delta^{13}C_{R\text{-soil}}$ as well as spatial variability in $\delta^{13}C_{R\text{-soil}}$ and $\delta^{13}C_{R\text{-foliage}}$.

[35] Our second hypothesis, that G_c influences $\delta^{13}C$ of respiratory fluxes of carbon, was supported by the measurements of $\delta^{13}C_R$ and $\delta^{13}C_{R\text{-soil}}$ (Figures 5a and 5b, Table 2). It is surprising that the relationship was even weakly significant between $\delta^{13}C_R$ and G_c (Figure 5a, $r^2 = 0.35$, $P = 0.04$) considering the limited range of $\delta^{13}C_R$. The $\delta^{13}C_{R\text{-soil}}$ results support a canopy-level control more strongly, both directly through the relationship with G_c and indirectly through the relationships with environmental factors known to affect G_c such as θ, PAR, and vpd (Table 2). However, T_{soil} was also well-correlated with $\delta^{13}C_{R\text{-soil}}$. This may be causal due to temperature regulation of rates and sources of belowground respiration, or it may be spurious due to the inherent relationship between soil drying and soil temperature. The $\delta^{13}C_{R\text{-foliage}}$ correlations provided a less intuitive set of results, with some supporting a temperature influence

(negative relationships with temperature) or supporting a positive effect of PAR (Table 2). To our knowledge, ours are the first isotopic measurements of foliar respiratory fluxes that have been conducted in a field setting; therefore we cannot compare our methodology or results to other studies at this time.

[36] The fact that $\delta^{13}C_{R\text{-}soil}$ exhibited relatively strong relationships with driving factors leads us to suggest that belowground carbon fluxes may be critical in regulating $\delta^{13}C_R$ variation. *Mortazavi and Chanton* [2002] made a similar conclusion in a study of a slash pine forest in southeastern United States, in which $\delta^{13}C_{R\text{-}soil}$ actually acted to buffer $\delta^{13}C_R$ from isotopic variation of aboveground respiration. However, the lack of consistency between $\delta^{13}C_{R\text{-}soil}$, $\delta^{13}C_{R\text{-}foliage}$, and $\delta^{13}C_R$, either real or due to methodological issues, makes strong conclusions about the controls over $\delta^{13}C_R$ in this study impossible.

[37] An interesting result is that correlations of $\delta^{13}C_{R\text{-}soil}$ with driving parameters have shorter lags than for $\delta^{13}C_{R\text{-}foliage}$ or $\delta^{13}C_R$ (Table 2). The average lag period for all parameters shown in Table 2 is 1.1 days for $\delta^{13}C_{R\text{-}soil}$, compared to 3.7 and 4.9 for $\delta^{13}C_{R\text{-}foliage}$ and $\delta^{13}C_R$. This is somewhat surprising given that foliage is located much closer to the source of assimilation than soil and hence should have a shorter delay between the time of assimilation and respiration if transport distance controls time lags. One possible interpretation is that the short time lags for $\delta^{13}C_{R\text{-}soil}$ may be due to a direct response of $\delta^{13}C_{R\text{-}soil}$ to changes in conditions at the soil level rather than via changes in canopy gas exchange. This would allow $\delta^{13}C_{R\text{-}soil}$ to respond immediately to meteorological changes as long as those forcing factors are transferred into changes in soil conditions. Indeed, $\delta^{13}C_{R\text{-}soil}$ was well correlated not only with factors that constrain canopy gas exchange, but it was also correlated with T_{soil} (Table 2). This may be spurious in that T_{soil} is likely to rise as θ and G_c decline, or it may be causal in that changes in T_{soil} could drive changes in the sources and certainly rates of respiration belowground. Unfortunately, comparison of the component fluxes is difficult because $\delta^{13}C_{R\text{-}foliage}$ appears to be coupled to different driving factors than $\delta^{13}C_{R\text{-}soil}$ and $\delta^{13}C_R$ (as shown in Table 2). Averaging periods did not differ substantially for the different components.

[38] The positive relationship between NEE and $\delta^{13}C_{R\text{-}soil}$ and $\delta^{13}C_{R\text{-}foliage}$ (Table 2) is consistent with the idea that periods of low assimilation (associated with low G_c) can cause isotopically enriched carbon isotope ratios of photo-assimilate and subsequent respiratory fluxes [*Randerson et al.*, 2002b]. There are at least two possible causes of these relationships: (1) NEE and $\delta^{13}C$ of photo-assimilate are indirectly correlated because both are directly linked to G_c, or (2) increased rates of ecosystem respiration are associated with isotopic changes in the carbon substrate used for respiration (i.e., a switch to more enriched substrates). There are a myriad of potential mechanisms for the second option including both autotrophic and heterotrophic tissues. However, the first option, that G_c causes simultaneous shifts in $\delta^{13}C$ of photo-assimilate and NEE is supported by the relationships between NEE, the isotopic content of respiratory CO_2, and G_c. Long-term data sets comparing $\delta^{13}C_R$, NEE and G_c will be necessary to further test the relationships and mechanisms controlling rates and signatures of carbon fluxes.

[39] The shorter time lag for the response of NEE to G_c (zero-day lag) than for either $\delta^{13}C_R$ to G_c (2-day lag, 1-day average, Table 2) or $\delta^{13}C_{R\text{-}soil}$ to G_c (1-day lag, 2-day average, Table 2) suggests that CO_2 flux rates and isotopic signatures are temporally decoupled. We suspect decoupling of rates and signatures is due to variation in within-ecosystem carbon transport after carbon assimilation. Future work on the relationships between fluxes and isotopic signatures should examine the controls and temporal variation of within-ecosystem carbon transport.

[40] A noteworthy result of this study is that none of the respiratory fluxes, including the foliar fluxes, isotopically matched the $\delta^{13}C$ of whole-leaf tissue (Figure 3). Leaf tissue $\delta^{13}C$ was 0.5 to 3.0‰ more negative than any of the observed fluxes, indicating an isotopic disequilibrium between stored and respired carbon. Similar results have been observed by *Pate and Arthur* [1998], *Ometto et al.* [2002], and *Pataki et al.* [2003], among others. A potential cause of this discrepancy is that the carbon in leaf tissue is predominately derived from photosynthesis during spring months when the climate is wet and mild and c_i/c_a is high (resulting in depleted $\delta^{13}C$). Our $\delta^{13}C$-flux measurements were conducted at least 1 month after the foliar carbon was assimilated, after conditions had become hotter and drier. Future work comparing $\delta^{13}C$ of stocks and fluxes would benefit from a time series analysis starting before bud break with repeated measurements throughout the period of leaf elongation.

[41] An important point must be made about the limited variation in $\delta^{13}C_R$ observed in this study. While it may be tempting to consider this result as evidence of constancy of $\delta^{13}C_R$, large variation in $\delta^{13}C_R$ at this site does occur, as observed between 1997 and 2000 [*Bowling et al.*, 2002] and as observed through weekly Keeling plots in 2001–2002 (N. G. McDowell et al., unpublished data, 2003: ~8.0‰ variation annually). At this site, variability in $\delta^{13}C_R$ is minimal during the rain-free summer period but is much larger during periods when precipitation is present, in the autumn, winter, and spring. We suspect the summer-period constancy in $\delta^{13}C_R$ observed in the current study and in the N. G. McDowell et al. unpublished data is due to groundwater access via deep rooting of the forest trees, which buffers the ecosystem from drought effects. Although we cannot yet conclude exactly what factors regulate $\delta^{13}C_R$, it is clear that periods of constancy as well as variability in $\delta^{13}C_R$ occur at this forest.

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